ZMYND11-related syndromic intellectual disability: 16 patients delineating and expanding the phenotypic spectrum

T. Michael Yates[1*], Morgan Drucker[2*], Angela Barnicoat[3], Karen Low[4], Erica H. Gerkes[5], Andrew E. Fry[6], Michael J Parker[1], Mary O'Driscoll[7], Perrine Charles[8], Helen Cox[7], Isabelle Marey[8], Boris Keren[8], Tuula Rinne[9], Meriel McEntagart[10], Vijaya Ramachandran[11], Suzanne Drury[11], Fleur Vansenne[5], Deborah A. Sival[12], Johanna C. Herkert[5], Bert Callewaert[13], Wen-Hann Tan[14†], Meena Balasubramanian[1,15†].

1. Sheffield Clinical Genetics Service, Sheffield Children's NHS Foundation Trust, Sheffield, UK.
2. Department of Pediatrics, Johns Hopkins University, Baltimore, MD, USA.
3. Northeast Thames Regional Genetics Service, Great Ormond Street Hospital for Children, London, UK.
4. Department of Clinical Genetics, St Michael's Hospital, Bristol, UK.
5. University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, the Netherlands.
6. Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK.
7. West Midlands Regional Clinical Genetics Service and Birmingham Health Partners Birmingham Women's Hospital NHS Foundation Trust, Birmingham, UK.
8. Département de Génétique, APHP, Hopital La Pitie Salpetriere, Paris, France
9. Department of Genetics, Radboud University Medical Center, Nijmegen, the Netherlands.
10. South West Thames Regional Genetics Centre, St. George's Healthcare NHS Trust, St. George's, University of London, London, UK.
11. Congenica Limited, Biodata Innovation Centre, Wellcome Genome Campus, Cambridge CB10 1DR
12. Department of Pediatrics, Beatrix Children's Hospital, University Medical Center Groningen, University Medical Center Groningen, Groningen, the Netherlands.
13. Center for Medical Genetics, Ghent University Hospital and Department of Biomolecular Medicine, Ghent University, Ghent, Belgium.
14. Division of Genetics and Genomics, Boston Children's Hospital, and Harvard Medical School, Boston, MA, USA.
15. Academic Unit of Child Health, Department of Oncology & Metabolism, University of Sheffield, UK.

*Joint first authors †Joint senior authors

Correspondence to:
Dr Meena Balasubramanian
Sheffield Clinical Genetics Service
Sheffield Children's NHS Foundation Trust
Western Bank, Sheffield S10 2TH
Ph- 0114 2717025, Fax- 0114 2737467, E-mail- meena.balasubramanian@nhs.net

This work utilises data from the DDD study, commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003]

Abstract

Pathogenic variants in *ZMYND11*, which acts as a transcriptional repressor, have been associated with intellectual disability, behavioural abnormalities and seizures. Only 11 affected individuals have been reported to-date, and the phenotype associated with pathogenic variants in this gene have not been fully defined.

Here, we present 16 additional patients with predicted pathogenic heterozygous variants in *ZMYND11,* including four individuals from the same family, to further delineate and expand the genotypic and phenotypic spectrum of *ZMYND11*-related syndromic intellectual disability. The associated phenotype includes developmental delay, particularly affecting speech, mild-moderate intellectual disability, significant behavioural abnormalities, seizures, and hypotonia. There are subtle shared dysmorphic features, including prominent eyelashes and eyebrows, depressed nasal bridge with bulbous nasal tip, anteverted nares, thin vermilion of the upper lip and wide mouth. Novel features include brachydactyly and tooth enamel hypoplasia.
Most identified variants are likely to result in premature truncation and/or nonsense mediated decay. Two *ZMYND11* variants located in the final exon - p.(Gln586*) (likely escaping nonsense-mediated decay) and p.(Cys574Arg) - are predicted to disrupt the MYND-type zinc finger motif and likely interfere with binding to its interaction partners. Hence, the homogeneous phenotype likely results from a common mechanism of loss-of-function.

Keywords: Gene Expression Regulation, Intellectual Disability, Seizures, Zinc Fingers, Behavioral Symptoms

Introduction

The chromosome 10p15.3 microdeletion syndrome is characterised by developmental delay (DD) and intellectual disability (ID), craniofacial dysmorphism, behavioural abnormalities, hypotonia, and seizures (DeScipio et al., 2013). Haploinsufficiency of *ZMYND11* (NCBI Gene ID: 10771) is believed to account for many of the features associated with chromosome 10p15.3 microdeletion (Tumiene et al., 2017).

ZMYND11 has been shown to act as a transcriptional repressor by inhibiting the elongation phase of RNA Polymerase II by recognizing histone modification present in transcribed regions, specifically H3K36 trimethylation (Wen et al., 2014).

In support of the critical role of *ZMYND11* in the chromosome 10p15.3 microdeletion syndrome, patients with *de novo* truncating variants in *ZMYND11* have a similar phenotype, including ID, seizures, and beavioural issues (Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Popp et al., 2017). In addition, missense variants in this gene have also been associated with ID and seizures, although there is a more severe phenotype in patients with specific variants, which may be related to a gain-of-function mechanism (Cobben et al., 2014; Moskowitz et al., 2016). A splice site variant has also been reported in a child with autism spectrum disorder (Iossifov et al., 2012). In total, 11 patients with pathogenic variants in *ZMYND11* (MIM# 616083) have been reported to date (Aoi et al., 2019; Cobben et al., 2014; Coe, Witherspoon, Rosenfeld, Van Bon, et al., 2014; Iossifov et al., 2012; Moskowitz et al., 2016; Popp et al., 2017).

Here, we present 16 previously unreported individuals with pathogenic variants in *ZMYND11*, including four from the same family. We further delineate and expand the genotype-phenotype correlations and phenotypic spectrum of *ZMYND11*-related intellectual disability.

Methods

All patients were ascertained after routine referral to their local Clinical Genetics service. Patients 1, 3, 5 and 8 were gathered through international collaboration using GeneMatcher (Sobreira, Schiettecatte, Valle, & Hamosh, 2016). Patients 2, 6, 7, 9, 11 and 12 were identified through the Wellcome Trust Deciphering Developmental Disorders study (Wright et al., 2015). Patients 13-15 were identified as affected relatives of patient 12. Patients 4, 10 and 16 were identified through personal communication. Exome sequencing was performed on all probands, with a trio approach on patients 1, 3, 5, 6, 9-12, and 16; a duo approach on patients 2, 4, 7, and 8, as DNA samples were only available from one parent.

Sanger sequencing only was used to ascertain the presence of the familial variant in patients 13-15, and all other patients had their *ZMYND11* variant confirmed using this method. All sequence variants were described with reference to *ZMYND11* transcript NM_006624.5. All variants were classified according to the American College of Medical Genetics (ACMG) guidelines (Richards et al., 2015). Further information is available in the supplemental data. Patient variants have been uploaded to either ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Global Variome shared LOVD http://www.lovd.nl, or DECIPHER (https://decipher.sanger.ac.uk).

Results

Molecular results

16 individuals (including 13 probands and two additional children of one affected mother) had a predicted pathogenic variant in *ZMYND11*. Of these, eight were *de novo*, one was inherited by three sibs from their affected mother, one was paternally-inherited, and three were of unknown inheritance. Ten variants were predicted to result in protein truncation, two were missense, and one affected a splice site (Table 1). None of the variants in this series were present in the gnomAD database (v2.1.1) (Karczewski et al., 2019). Of the two missense variants, one was located in a zinc finger domain (c.1720T>C; p.(Cys574Arg)), and the other was not in a known functional domain (c.1246G>A; p.(Glu416Lys)). Further information is available in the supplemental data.

Patient phenotypes

Phenotypic information for all patients is shown in Table 1 In-depth patient summaries are available in the supplemental data (Supp. Patient Summaries). Prominent phenotypic features are detailed below. The denominators refer to the number of patients for whom the specific information is available.

Birth weight was at or above the 98th centile in three patients (3/14; 21%). Feeding problems (e.g. excess

vomiting after feeds, bottle feeding requiring more than one hour), were present in 6/13 patients (46%). Most patients had normal growth parameters and head circumference.

Development was delayed in all patients (14/14; 100%). The median age at independent walking was 24 months (with age range of 17 months to four years). Three patients remained unable to walk at the ages of two-and-a-half (for two individuals) and four years, respectively. Speech delay was prominent, with 14/14 (100%) affected. First words were achieved at a median age of two-and-a-half years (with age range of two years to four years). Two patients were non-ambulatory, and had not achieved speech at two-and-a-half and four years age respectively (2/14; 14%). All patients had mild to moderate intellectual disability (13/13; 100%).

Almost all patients had behavioural issues (14/16; 88%). These include attention deficit, hyperactivity and impulsivity (8/16; 50%), aggressive behaviour (8/16; 50%), and autism spectrum disorder or autistic traits (3/15; 20%). Neurological abnormalities were detected in 10/16 (63%); mostly hypotonia (5/16; 31%) and epilepsy (5/16; 31%).

Photographs of patients in this series are shown in Fig. 1. Dysmorphic facial features were judged to be present in 11/16 (69%). There were a number of shared facial features, including thick eyebrows, prominent eyelashes, depressed nasal bridge with bulbous nose, anteverted nares, thin vermilion of the upper lip and wide mouth.

Patients 12-14 in this series inherited their *ZMYND11* variant from their mother (patient 15). All individuals in this family had special educational needs; two of the siblings are now in employment. The *ZMYND11* variant found in Patient 9 was paternally inherited. Detailed phenotypic information is not available for the father.

Discussion

Here, we present 16 new individuals with predicted pathogenic variants in *ZMYND11*. Comparison with all previously published patients allows further delineation of the phenotypic spectrum associated with mutations in this gene (Table 2) (Supp. Table S1) (Aoi et al., 2019; Cobben et al., 2014; Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Iossifov et al., 2012; Moskowitz et al., 2016; Popp et al., 2017).

All patients (including our series) had developmental delay, particularly affecting speech, and ID. The severity of ID in this series is mild to moderate, but four patients have previously been described with severe ID (Cobben et al., 2014; Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Moskowitz et al., 2016; Popp et al., 2017). Behavioural issues are also a prominent feature both in our series and in those previously published (Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Popp et al., 2017) , including aggression, attention deficit/hyperactivity, and autism/autistic traits. Therefore, this series provides further evidence that behavioural abnormalities are a significant part of the *ZMYND11*-associated phenotype. These behavioural problems may pose a substantial psychosocial burden, especially if the intellectual disability is mild. Hypotonia and epilepsy affect 48% and 39% of all patients, respectively (including our series). This enables us to indisputably establish hypotonia and epilepsy as part of the phenotype associated with this syndrome.

Dysmorphic features, particularly thick eyebrows, prominent eyelashes and a bulbous nose, are present in the majority of patients (Fig. 1). These are in line with the patients reported by Coe et al., (2014). These dysmorphisms may prove useful with regard to reverse phenotyping. Feeding difficulties were present in 59% of all patients (including our series), although only three patients required supplementary feeding.

Brachydactyly, seen in two patients in our series, is a possible novel feature. Interestingly, tooth enamel hypoplasia, present in one patient in our series, has previously been reported in another patient with a *ZMYND11* variant (Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014), indicating this may be a rare and/or overlooked phenotypic feature, although formal dental assessment has not been documented for most patients.

In this series, three individuals inherited a predicted pathogenic *ZMYND11* variant from their affected mother; another patient inherited the pathogenic variant from his father on whom detailed phenotypic information was lacking. Inheritance of a pathogenic *ZMYND11* variant from an affected parent has been previously reported (Coe et al. 2014). Familial inheritance should therefore be considered in variant filtering and interpretation and reproductive counselling.

The majority of patients, including those in our series, have truncating variants, which are likely subject to nonsense-mediated decay and hence, result in haploinsufficiency (Fig. 2). Of note, the p.(Gln586*) variant in our series is located in the last exon and therefore may escape nonsense-mediated decay. The p.(Cys574Arg) variant is similarly located in the last exon. These variants may be expected to have a deleterious effect through disruption of the MNYD-type zinc finger motif. This motif interacts with a number of intracellular partners, for example the NCoR transcriptional corepressor (Masselink & Bernards, 2000), and amino acid variation within this motif has been shown to disrupt binding of these partners, resulting in reduced efficacy of *ZMYND11*-mediated transcriptional repression (Kateb et al., 2013; Masselink & Bernards, 2000). We suspect, therefore, that the two variants affecting the MYND-type zinc finger motif domain in our series will at least result in a reduced function of the protein. The phenotype of these patients and a previously reported individual (Coe et al. 2014) with a p.(Gln587del) variant in this motif is not notably different to those patients harbouring variants causing haploinsufficiency, supportina loss-of-function mechanism. The p.(Glu416Lys) variant in this series is not in a functional domain. It has been classified as likely pathogenic given that it is *de novo* and not present in the gnomAD database; however further research is required to determine the effect of this variant.

In contrast, two missense pathogenic variants have been reported in patients with notably different phenotypes to those in this series. The p.(Ser421Asn) variant resulted in a severe Angelman-like phenotype, and the p.(Arg600Trp) variant caused distinct facial dysmorphism, moderate to severe intellectual disability, and short stature (Cobben et al., 2014; Moskowitz et al., 2016). Given these distinct

phenotypes, it is possible that other mechanisms, including a gain-of-function, may be at play, but further research is required to characterise the effects of these specific variants.

Conclusions

We present a series of 16 patients with predicted pathogenic *ZMYND11* variants, predicted to result in haploinsufficiency or reduced protein function, together with a review of the published literature, allowing further delineation of the associated phenotype. Developmental delay and ID, usually mild to moderate, are universally present. Behavioural issues are frequent, and hypotonia and seizures are common. Feeding difficulties occur, but are usually mild. Subtle dysmorphism includes prominent eyelashes and eyebrows. Novel features include brachydactyly and tooth enamel hypoplasia. Our data will contribute to successful reverse phenotyping following genomic sequencing.

Data Availability Statement

Patient variants have been uploaded to either ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Global Variome shared LOVD http://www.lovd.nl, or DECIPHER (https://decipher.sanger.ac.uk). The accession numbers are: RCV000627377.1 (Clinvar), SGS 306759, SGS 307296, CAR 279594, SMB 307553, BWH 264849, GSH 282655 (DECIPHER) and via https://databases.lovd.nl/shared/genes/ZMYND11 (LOVD).

Acknowledgements

We would like to thank the families involved for their permission to publish this work. The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003]. This study makes use of DECIPHER (http://decipher.sanger.ac.uk), which is funded by Wellcome. See Nature PMID: 25533962 or www.ddduk.org/access.html for full acknowledgement. BC is a Senior Clinical Investigator of the Research Foundation – Flanders.

Conflict of Interest

The authors do not have any conflict of interest to disclose.

Consent

Informed consent was obtained for all subjects for inclusion in this study.

References

Aoi, H., Mizuguchi, T., Ceroni, J. R., Kim, V. E. H., Furquim, I., Honjo, R. S., … Matsumoto, N. (2019). Comprehensive genetic analysis of 57 families with clinically suspected Cornelia de Lange syndrome. *Journal of Human Genetics*, *64*(10), 967–978. https://doi.org/10.1038/s10038-019-0643-z

Cobben, J. M., Weiss, M. M., van Dijk, F. S., De Reuver, R., de Kruiff, C., Pondaag, W., … Yntema, H. G. (2014). A de novo mutation in ZMYND11, a candidate gene for 10p15.3 deletion syndrome, is associated with syndromic intellectual disability. *European Journal of Medical Genetics*, *57*(11–12), 636–638. https://doi.org/10.1016/j.ejmg.2014.09.002

Coe, B. P., Witherspoon, K., Rosenfeld, J. A., van Bon, B., Vulto-van Silfhout, A., Bosco, P., … Pettinato, R. (2014). Refining analyses of copy number variation identifies specific genes associated with developmental delay. *Nature Genetics*, *46*(10), 1063–1071. https://doi.org/10.1038/ng.3092

Coe, B. P., Witherspoon, K., Rosenfeld, J. A., Van Bon, B. W. M., Vulto-Van Silfhout, A. T., Bosco, P., … Eichler, E. E. (2014). Refining analyses of copy number variation identifies specific genes associated with developmental delay. *Nature Genetics*, *46*(10), 1063–1071. https://doi.org/10.1038/ng.3092

DeScipio, C., Conlin, L., Rosenfeld, J., Tepperberg, J., Pasion, R., Patel, A., … Hilhorst-, Y. (2013). Subtelomeric Deletion of Chromosome 10p15.3: Clinical Findings and Molecular Cytogenetic Characterization. *American Journal of Medical Genetics Part A*, (9), 2152–2161. https://doi.org/10.1002/ajmg.a.35574.

Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., … Wigler, M. (2012). De Novo Gene Disruptions in Children on the Autistic Spectrum. *Neuron*, *74*(2), 285–299. https://doi.org/10.1016/j.neuron.2012.04.009

Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., … MacArthur, D. G. (2019). Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. *BioRxiv*, 531210. https://doi.org/10.1101/531210

Kateb, F., Perrin, H., Tripsianes, K., Zou, P., Spadaccini, R., Bottomley, M., … Sattler, M. (2013). Structural and Functional Analysis of the DEAF-1 and BS69 MYND Domains. *PLoS ONE*, *8*(1). https://doi.org/10.1371/journal.pone.0054715

Masselink, H., & Bernards, R. (2000). The adenovirus E1A binding protein BS69 is a corepressor of transcription through recruitment of N-CoR. *Oncogene*, *19*(12), 1538–1546. https://doi.org/10.1038/sj.onc.1203421

Moskowitz, A. M., Belnap, N., Siniard, A. L., Szelinger, S., Claasen, A. M., Richholt, R. F., … Schrauwen, I. (2016). A de novo missense mutation in ZMYND11 is associated with global developmental delay, seizures, and hypotonia . *Molecular Case Studies*, *2*(5), a000851.

https://doi.org/10.1101/mcs.a000851
Popp, B., Ekici, A. B., Thiel, C. T., Hoyer, J., Wiesener, A., Kraus, C., … Zweier, C. (2017). Exome Pool-Seq in neurodevelopmental disorders. *European Journal of Human Genetics*, *25*(12), 1364–1376. https://doi.org/10.1038/s41431-017-0022-1
Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., … Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30
Sobreira, N., Schiettecatte, F., Valle, D., & Hamosh, A. (2016). GeneMatcher: A Matching Tool for Connecting Investigators with an Interest in the Same Gene. *Human Mutation*, *36*(10), 928–930. https://doi.org/10.1002/humu.22844.
Tumiene, B., Čiuladaitė, Preikšaitienė, E., Mameniškienė, R., Utkus, A., & Kučinskas, V. (2017). Phenotype comparison confirms ZMYND11 as a critical gene for 10p15.3 microdeletion syndrome. *Journal of Applied Genetics*, *58*(4), 467–474. https://doi.org/10.1007/s13353-017-0408-3
Wen, H., Li, Y., Xi, Y., Jiang, S., Stratton, S., Peng, D., … Shi, X. (2014). ZMYND11 links histone H3.3 K36 trimethylation to transcription elongation and tumor suppression. *Nature*, *508*(7495), 263–268. https://doi.org/10.1038/nature13045
Wright, C. F., Fitzgerald, T. W., Jones, W. D., Clayton, S., McRae, J. F., Van Kogelenberg, M., … Firth, H. V. (2015). Genetic diagnosis of developmental disorders in the DDD study: A scalable analysis of genome-wide research data. *The Lancet*, *385*(9975), 1305–1314. https://doi.org/10.1016/S0140-6736(14)61705-0

Figure Legends

Figure 1.

Photographs of patients in this series. Patient ages are as follows (y- years; mo – months): 1 – 3y, 2 – 8y, 3 – 5y 10mo, 4 – 8y, 6 – 4y, 7 – 13y 8mo, 9 – 8y, 10 – 2y 7mo, 11 – 15y, 12 – 17y, 13 – 22y, 14 – 20y, 15 – 47y, 16 – 2.5y. Note shared dysmorphic features (particularly in patients 1, 3-14 and 16) including prominent eyelashes and flattened nasal bridge with bulbous nasal tip.

Figure 2.

ZMYND11 protein showing pathogenic variants in this series (below protein) and previously reported (above protein) (Cobben et al., 2014; Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Iossifov et al., 2012; Moskowitz et al., 2016; Popp et al., 2017) (transcript NM_006624.5, Human Genome Build GRCh37.p13). Functional domains are labelled according to their location in the protein. The tandem

PWWP (Pro-Trp-Trp-Pro)-Bromo domains function in recognising H3K36 trimethylation.

Proposed pathogenic mechanism	**Haploinsufficiency: NMD**										
Patient no	1	2	3	4	5	6	7	8	9	10	**Total**
DECIPHER ID		SGS 306759				SGS 307296	CAR 279594		SMB 307553		
ZMYND11 variant	c.46C>T	c.117-2A>T	c.630C>G	c.705_708delTGAG	c.1089G>A	c.1129del	c.1315_1318del	c.1525_1526del	c.1531C>T	c.1572dup	
Predicted effect on protein	p.(Gln16*)	Splice acceptor variant	p.(Tyr210*)	p.(Glu236Lysfs*52)	p.(Trp363*)	p.(Ser377Profs*11)	p.(Thr440Argfs*3)	p.(Lys509Glufs*6)	p.(Gln511*)	p.(Asp525Glyfs*5)	
Inheritance	de novo	unknown	de novo	de novo	de novo	de novo	unknown	unknown	pat	de novo	
Pathogenicity (ACMG criteria)	Pathogenic (PVS1, PS2, PM2, PP3)	Pathogenic (PVS1, PM2, PP3)	Pathogenic (PVS1, PS2, PM2, PP3)	Pathogenic (PVS1, PS2, PM2)	Pathogenic (PVS1, PS2, PM2, PP3)	Pathogenic (PVS1, PS2, PM2, PP3)	Likely pathogenic (PVS1, PM2)	Likely pathogenic (PVS1, PM2)	Pathogenic (PVS1, PM2, PP3)	Likely Pathogenic (PM2, PVS1_S, PS2_M)	
Age reported	3y	8y	5y 10 mo	8y	8y	4y	13y 8mo	18y	8y	2y 7mo	
Gender	F	F	F	M	M	M	M	M	M	F	
Feeding problems	Yes	No	nd	Yes (NG supplementation)	nd	No	Yes (NG supplementation)	Yes	Yes	No	5/8 (63%)
Dysmorphic	Yes	No	Yes	No	Yes	No	Yes	No	Yes	Yes	6/10 (60%)
Delayed development	Yes	nd	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9/9 (100%)
Gross motor delay	2y	17 mo	2y	2y	2y	Cannot walk unaided 4y	22 mo	18 mo	2-2.5y	Not yet achieved	8/10 (80%)
Speech delay	Limited vocabulary, difficult to understand	nd	Short sentences at 4y	2y	First words 3y; 2-word phrases 3.5y	Not yet achieved	2y; difficult to understand until 3y	4y	2-2.5y	2y	9/9 (100%)
ID	nd	Mild, mainstream school with extra help	Mild	Mild	Mild	Mild	Moderate	nd	Mild, mainstream school with extra help. Dyspraxic.	Moderate	8/8 (100%)
Behavioural difficulties	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	9/10 (90%)
Attention deficit/ hyperactivity/ impulsivity	Yes	Yes	No	Yes	No	No	Yes	Yes	No	No	5/10 (50%)
Aggression/ anger	No	No	Yes	No	Yes	No	Yes	No	Yes	No	4/10(40%)
Autism/ autistic traits	No	No	Yes	No	No	No	No	No	Yes	No	2/10 (20%)
Hypotonia	Yes	No	No	Yes	No	No	No	Yes	Yes	No	4/10 (40%)
Epilepsy	No	Yes	Yes	No	No	No	No	No	No	No	2/10 (20%)

Proposed pathogenic mechanism	**Predicted to disrupt MYND zinc-finger domain**						**Missense**	**Overall Total**
Patient no	11	12	13	14	15	**Total**	16	
DECIPHER ID	BWH 264849	GSH 282655						
ZMYND11 variant	c.1720T>C	c.1756C>T	c.1756C>T	c.1756C>T	c.1756C>T		c.1246G>A	
Predicted effect on protein	p.(Cys574Arg)	p.(Gln586*)	p.(Gln586*)	p.(Gln586*)	p.(Gln586*)		p.(Glu416Lys)	

Inheritance	de novo	mat	mat	mat	unknown		de novo	
Pathogenicity (ACMG criteria)	Likely pathogenic (PS2, PM2, PP3)	Pathogenic (PVS1, PM2, PP3)	Pathogenic (PVS1, PM2, PP3)	Pathogenic (PVS1, PM2, PP3)	Pathogenic (PVS1, PM2, PP3)		Likely pathogenic (PS2, PM2)	
Age reported	15y	17y	22y	20y	47y		2y 5 mo	
Gender	M	M	F	F	F		M	
Feeding problems	No	No	No	No	nd	0/4 (0%)	Yes	5/13 (38%)
Dysmorphic	Yes	Yes	Yes	Yes	No	4/5 (80%)	Yes	11/16 (69%)
Delayed development	Yes	Yes	Yes	Yes	nd	4/4 (100%)	Yes	14/14 (100%)
Gross motor delay	13 mo	2.5 y	18 mo	4y	nd	2/4 (50%)	Not yet achieved	11/15 (73%)
Speech delay	2-2.5y	4-5y	First words around 4y	First words around 4y	nd	4/4 (100%)	Not yet achieved	13/13 (100%)
ID	Moderate ID, attends special school	Yes, attended special school, now in simple employment	Yes, attended special school, not able to take GCSE, volunteering activities in school, currently in college	Yes, attended special school, not able to take GCSE but currently working in retail	Yes, attended special school	5/5 (100%)	nd	13/13 (100%)
Behavioural difficulties	Yes	Yes	Yes	Yes	No	4/5 (80%)	Yes	14/15 (93%)
Attention deficit/ hyperactivity/ impulsivity	Yes	Yes	Yes	No	No	3/5 (60%)	No	8/16 (50%)
Aggression/ anger	Yes	Yes	Yes	Yes	No	4/5 (80%)	No	8/16 (50%)
Autism/ autistic traits	Yes	No	No	No	No	1/5 (20%)	nd	3/15 (20%)
Hypotonia	No	No	No	No	No	0/5 (0%)	Yes	5/16 (31%)
Epilepsy	Yes	No	Yes	No	Yes (as child)	3/5 (50%)	No	5/15 (33%)

Table 1. Genotypic and phenotypic data for patients in this series, ordered according to likely pathogenic mechanism. Totals include only those patients for whom the presence or absence of the feature is reported. Mutation nomenclature according to Human Genome Variation Society (HGVS) recommendations (http://varnomen.hgvs.org/). All variants were analysed according to transcript NM_006624.5. American College of Medical Genetics and Genomics sequence interpretation criteria according to Richards et al., 2015.

Proposed pathogenic mechanism	**Haploinsufficiency:NMD**								**Disruption of MYND zinc-finger domain**	
Patient no	Iossifov et al.	Coe et al. ***Nijmegen DNA05-04370***	Coe et al. ***Adelaide 3553***	Coe et al. **Nijmegen DNA-017151**	Coe et al. **Nijmegen DNA-002424**	Coe et al. **Nijmegen DNA-013587**	Popp et al.	Aoi et al.	Coe et al. ***Adelaide 20124***	**Total (wher documen**
ZMYND11 variant	c.1159-1G>A	c.1246_1247del	c.454_455insC	c.206dup	c.976C>T	c.561del	c.383del	c.1438del	c.1759_1761del	
Predicted effect on protein	Splice variant	p.(Glu416Serfs*5)	p.(Asn152Thrfs*26)	p.(Thr70Asnfs*12)	p.(Gln326*)	p.(Met187Ilefs*19)	p.(Ser128Leufs*42)	p.(Asp480Thrfs*3)	p.(Gln587del)	
Type of predicted variant effect	Splice variant	Frameshift	Frameshift	Frameshift	Nonsense	Frameshift	Frameshift	Frameshift	In-frame	
Feeding problems	nd	nd	nd	nd	nd	nd	Yes	nd	Yes	2/2 (100
Dysmorphic	nd	Yes	No	Yes	Yes	Yes	nd	Yes	Yes	6/7 (86
Gross motor delay	nd	nd	Yes	Yes	nd	Yes	nd	nd	nd	3/3 (100
Speech delay	nd	Nonverbal	Yes	Yes	Yes	Yes	nd	nd	Yes	6/6 (100
ID	nd	Severe	nd	Mild	Mild	Mild	Severe	Yes	Mild	7/7 (100
Behavioural difficulties	nd	nd	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7/7 (100
Attention deficit/ hyperactivity/ impulsivity	nd	nd	nd	No	No	Yes	nd	No	nd	1/4 (25
Aggression/ anger	nd	nd	nd	No	nd	Yes	Yes	No	nd	2/4 (50
Autism/ autistic traits	Yes	Yes	nd	Yes	nd	No	nd	No	No	3/6 (50
Neurological abnormality	nd	Yes	Yes	No	nd	No	Yes	nd	Yes	4/6 (67
Hypotonia	nd	Yes	No	Yes	nd	nd	Yes	nd	Yes	4/5 (80
Epilepsy	nd	Yes	Yes	No	nd	No	Yes	nd	No	3/6 (50

Table 2. Genotypic and phenotypic data for all previously reported patients with *ZMYND11* variants, ordered according to likely pathogenic mechanism, with summary total including this series. Totals include only those patients for whom the presece or absence of the feature is reported. Mutation nomenclature according to Human Genome Variation Society (HGVS) recommendations (http://varnomen.hgvs.org/). All variants were analysed according to transcript NM_006624.5. American College of Medical Genetics and Genomics sequence interpretation criteria according to Richards et al., 2015.

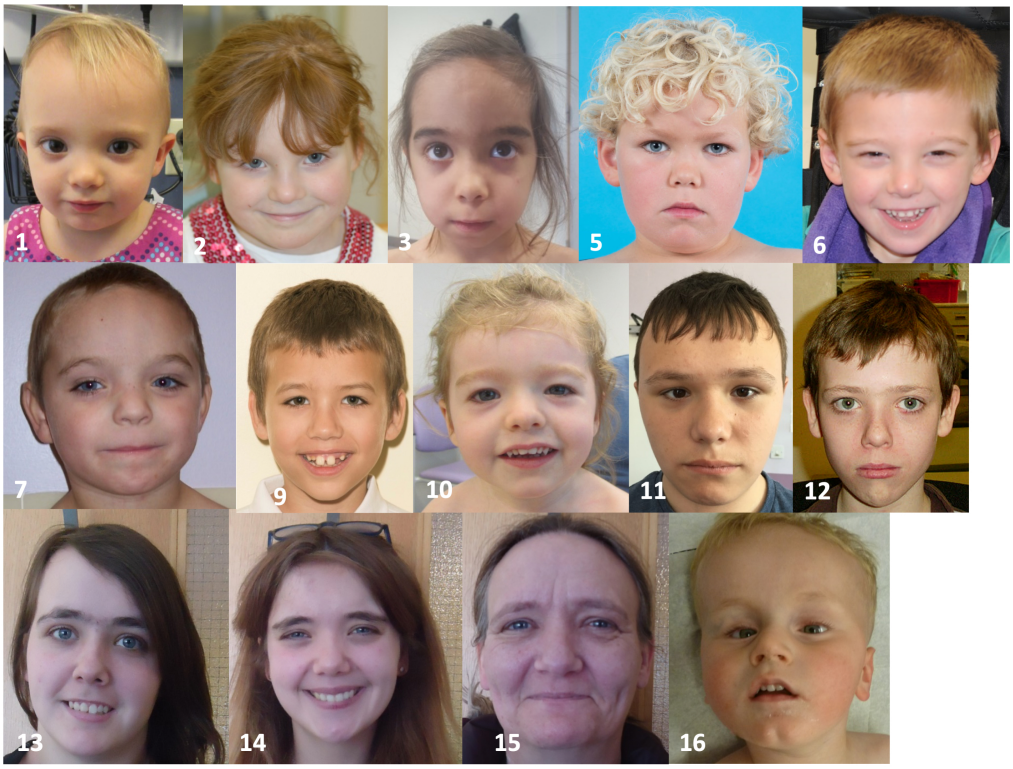

Figure 1: Photographs of patients in this series. Patient ages are as follows (y- years; mo – months): 1 – 3y, 2 – 8y, 3 – 5y 10mo, 4 – 8y, 6 – 4y, 7 – 13y 8mo, 9 – 8y, 10 – 2y 7mo, 11 – 15y, 12 – 17y, 13 – 22y, 14 – 20y, 15 – 47y, 16 – 2.5y. Note shared dysmorphic features (particularly in patients 1, 3-14 and 16) including prominent eyelashes and flattened nasal bridge with bulbous nasal tip.

253x190mm (300 x 300 DPI)

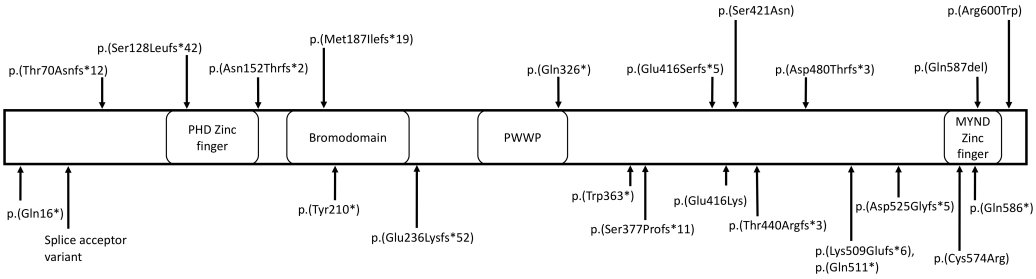

Figure 2: ZMYND11 protein showing pathogenic variants in this series (below protein) and previously reported (above protein) (Cobben et al., 2014; Coe, Witherspoon, Rosenfeld, van Bon, et al., 2014; Iossifov et al., 2012; Moskowitz et al., 2016; Popp et al., 2017) (transcript NM_006624.5, Human Genome Build GRCh37.p13). Functional domains are labelled according to their location in the protein. The tandem PWWP (Pro-Trp-Trp-Pro)-Bromo domains function in recognising H3K36 trimethylation.

338x190mm (400 x 400 DPI)

Patient 1

This 3-year-old female is the only child of non-consanguineous Caucasian parents. There is no family history of developmental delay.

She was born at 37 weeks' gestation following a pregnancy complicated by hyperemesis gravidarum, subchorionic hemorrhage, and nuchal cord x 2. Birth weight was 2400 g. She was born with congenital bilateral hip dysplasia, which resolved with a Pavlik harness. She was noted to have hypotonia since birth and had difficulty with feeding during the first three months of life, requiring high-caloric formula supplementation in addition to breastfeeding.

She was evaluated by Early Intervention services at 6 months of age due to global developmental delay and qualified for speech and physical therapy. She sat independently at age 8-9 months, which coincided with the time her leg braces were removed, walked independently at age 2 years, and climbed stairs assisted by a railing at age 3 years. Age at first words is unknown; however, she spoke ~24 words with some 2-word phrases at 2 years, though speech remains mostly unintelligible to strangers at age 3 years. Receptive language is better than expressive language.

Socially, she is an interactive and happy child. She has exhibited significant interest in objects, but there is no concern for restricted interests. She does exhibit biting and hitting behavior when frustrated and occasional hand-flapping behavior when in unfamiliar situations; however, there is no overall concern for a behavioral disorder.

Physical exam at age 3 years and 2 months showed a height of 93.4 cm (-0.49 SD), weight of 13.9 kg (-0.21 SD), and occipitofrontal head circumference (OFC) of 48.3 cm (25^{th} percentile). She had generalized hypotonia and bilateral fifth finger clinodactyly. Facial dysmorphisms included micrognathia, widow's peak, mild hypertelorism, small earlobes, broad single uvula, high palate, and thin vermilion of upper lip.

Previous investigations included normal 46,XX karyotype and negative chromosome 15q methylation studies. Chromosomal microarray demonstrated an area of homozygosity on chromosome 6p12.1-q12 (hg19 coordinates: 55,884,356-67,452,305).

Patient 2

This 8-year old child is the daughter of non-consanguineous parents. Neither the parents nor siblings exhibit a phenotype similar to the patient's.

She was born at 42 weeks' gestation following an uncomplicated pregnancy. Birth weight was 3.1 kg. There were no neonatal complications and no feeding difficulties.

Developmental milestones were globally delayed. At the age of 7.5 years, she had mild intellectual disability (ID) and was two to three years behind her expected levels in some subjects. She attended a mainstream school with additional learning support.

She displayed impulsive behavior, attention difficulties, hyperactivity and had significant mood swings. She also had episodes of shaking her head side-to-side occurring usually in bright sunlight, which were thought to be non-epileptic in nature.

She had epilepsy with seizures involving vacant staring episodes and rolling of the eyes upward. Focal and generalized abnormalities were noted on EEG. Cranial MRI scan was normal.

Physical exam at age five years demonstrated OFC 51.5 cm. She did not show any facial dysmorphisms.

Patient 3

This 5 years and 10 month old female was born at term after an uncomplicated pregnancy. Birth weight was 3.25kg. There were no neonatal complications. Neither the parents nor siblings exhibit a phenotype similar to the patient's.

Developmental milestones have been delayed, with the patient sitting at 12 months and walking at 24 months. Language development was delayed and she had three word sentences at four years age. She had temper tantrums and was also diagnosed as having an autism spectrum disorder. She had epilepsy, and frontotemporal spikes were seen on EEG.

Physical exam at age 4 years 7 months demonstrated height 107cm, weight 17.35kg and OFC 51cm. Facial dysmorphisms include scaphocephaly, prominent forehead, synophrys, upslanting palpebral fissures, broad nasal bridge, bubous nasal tip, mild epicanthal folds, flat philtrum and small chin. She also had 5^{th} finger clinodactyly and short fingers.

Chromosomal microarray testing was normal.

4 FV

This 8-year old child is the son of non-consanguineous parents. The parents do not have a similar phenotype as the patient.

He was born at 36 weeks via induced delivery due to reduced fetal movements. Birth weight was 1.98 kg. The delivery was complicated by perinatal asphyxia, and he had apnoeic episodes over the first 24 hours. He was admitted to Neonatal Intensive care and required nasogastric feeding.

Developmental milestones have been delayed. Social smile was at 11 weeks age and sitting independently was achieved at 12 months. Walking independently and first words were achieved at two years of age.

At the age of eight years, he had mild intellectual disability. He had a short attention span, and anxiety. He had an ataxic gait and myoclonic jerks affecting the face, shoulders, arms, and hands. He was also noted to have axial hypotonia, a dystonic posture of the feet during walking, excess drooling and slurred speech. Cranial MRI demonstrated perirolandic atrophy, a small area of increased signal in the right thalamus, small thalami, and secondary hypomyelination due to cortical damage in both internal capsules, affecting the left more than right. His phenotype was not thought to be explained by perinatal asphyxia.

Physical exam at age seven years demonstrated height 123.9 cm, weight 24.6 kg, and OFC 52 cm. He was not thought to be dysmorphic, although he did have long eyelashes.

Patient 5

This 8-year-old child is the son of non-consanguineous parents. Neither the parents nor siblings exhibit a phenotype similar to the patient's.

He was born at 38+4 weeks' gestation following a pregnancy complicated by maternal cholecystitis treated by cholecystectomy. Prenatal ultrasound demonstrated a hyperechogenic structure in the right wall of the ventricle. Birth weight was 2.9 kg. There were no neonatal complications, and cardiac ultrasound after birth was normal.

Developmental milestones have been delayed, with the patient sitting independently late, walking independently at 2 years, and having first words at 3 years. He developed 2-word phrases at 3 years and 6 months. Additionally, socio-emotional development is significantly delayed, and he exhibits aggressive behavior. Socio-emotional developmental age of 2 years at calendar age of 8 years. TIQ is 84.

Additionally, the patient has had progressive obesity since childhood. He also exhibited sudden-onset double vision at 7 years with no other neurological abnormalities and normal cranial MRI. At that time, he was already known to have anisometropy and hypermetropy.

Physical exam at age 6 years demonstrated height at 0 SD and weight at +3 SD, with OFC at +1.7 SD at age 7 years and10 months. Facial dysmorphisms include round face with periorbital fullness and esotropia, low and broad nasal bridge with epicanthal folds, and full nasal tip. He also has mild brachydactyly of the hands and feet, mild hypermobility, and toe nails that break easily and fragile enamel of teeth.

Previous investigations included testing for a panel of 500 genes associated with intellectual disability (ID) (*ZMYND11* not yet included). Chromosomal microarray showed a mat7q36.1 dup (including the *KMT2C* and *FABP5P3* genes), which was not thought to explain the patients' phenotype.

Patient 6

This 4-year old child is the son of non-consanguineous parents. Neither the parents nor siblings exhibit a phenotype similar to the patient's.

He was born at 40 weeks' gestation after an uncomplicated pregnancy. Birth weight was 2.98kg. There were no neonatal complications and no feeding difficulties.

Developmental milestones have been delayed, with social smile at 14 weeks of age and sitting independently at 16 months of age. Walking independently and speech were not yet achieved at the age of four years.

He had two generalized non-febrile seizures at the age of four years. Cranial MRI demonstrated bilateral frontoparietal polymicrogyria.

Physical exam at age two years demonstrated length 80cm, weight 9.6kg, and OFC 45cm. He was not thought to have dysmorphic features.

Previous testing of a panel of genes associated with cortical malformation was normal.

Patient 7

This 13 years and 8 month old child is the son of non-consanguineous parents. His mother was thought to have some shared phenotypic features, although it is not known if she carries the same *ZMYND11* variant.

He was born at 42 weeks' gestation, and there was thought to be maternal alcohol and recreational drug use during pregnancy. Birth weight was 3.54kg. He was discharged from hospital soon after birth but required readmission at one month of age due to excessive vomiting after feeding. He needed gastrostomy feeding at that time.

Developmental milestones have been delayed, with the patient sitting independently at 10 months of age, walking independently at 22 months age, and developing first words at 2 years of age, although these were difficult to understand until 3 years

Physical exam at age 13 years and 8 months demonstrated height 155.6cm, weight 46.5kg and OFC 53.5cm. Facial dysmorphisms included round face, hypertelorism, thin top lip, underdeveloped nasal alae, clinodactyly of the 5th finger and down-sloping shoulders. At 3 years of age he had a prominent metopic ridge, which became less prominent over time. He had challenging hyperactive, aggressive and impulsive behavior, and he was diagnosed with attention deficit hyperactivity disorder at 11 years.

Previous testing including chromosomal microarray and Fragile X was normal.

Patient 8

This 18-year-old male was born to non-consanguineous parents. Neither parents nor siblings had a similar phenotype; a maternal cousin was reported to have autistic features and developmental delay.

He was born at 39 weeks' gestation, after a pregnancy complicated by placental abruption. Birth weight was 3.38kg. There was no requirement for Special Care, although he was noted to have feeding difficulties. Developmental milestones have been delayed, with the patient walking independently at 18 months and achieving first words at four years of age.

The patient developed a hyperactivity disorder, as well as spinal kyphosis and axial hypotonia. Cranial MRI was normal, and EEG showed background disorganization with no epileptic activity. Physical exam at age 18 years demonstrated height 178cm, weight 73kg and OFC 57.5cm. He had a supernumerary nipple, and was not thought to be dysmorphic.

Previous testing including chromosomal microarray, Myotonic Dystrophy Type 1 and Fragile X was normal.

Patient 9

This 8-year old child is the son of non-consanguineous parents. His father had macrocephaly.

He was born at 42 weeks' gestation after an uncomplicated pregnancy. Birth weight was 4.76kg. There were some early feeding difficulties.

Developmental milestones have been delayed, with the patient sitting independently at 13 months. Independent walking and first words were achieved at 2.5 years of age.

At eight years of age, he had mild ID and was dyspraxic. He attended a mainstream school with extra help. He had episodes of aggressive behaviour, which could be difficult to manage. He had autistic traits but did not meet the criteria for a formal diagnosis of autism spectrum disorder. Cranial MRI scan demonstrated ventriculomegaly.

Physical exam at age three years demonstrated height 104.8 cm, weight 18.9kg and OFC 55.5cm. Facial dysmorphisms included epicanthus, long eyelashes, heterochromia and macrocephaly. There was a single black macule on the scalp. The patient also had pectus excavatum, clinodactyly of the 5th finger, pes planus, hypotonia and joint hypermobility.

Previous sequencing of the *KDM6A* and *KMT2D* genes was normal.

10

This two year seven month old child was noted to be small for gestational age on prenatal scans. She was born at 39+6 weeks gestation with a birth weight of 2.35kg (<0.4th centile). She did not require Special Care and did not have any feeding problems.

Developmental milestones have been delayed. Social smile was at eight to nine weeks, sitting independently at 11 months and she was cruising at two years of age. At two-and-a-half years age she was not walking confidently on her feet, although she did walk on her knees. After two years of age, she could say 'up' and 'no'. At two-and-a-half years age, she could recognize animal names, and could follow some simple one stage commands.

She was thought to be a generally placid child, but could get frustrated when not understood. She can express her wants with gestures or vocalisations, to some degree.

Physical exam at two years and seven months of age demonstrated height on the 2nd centile, weight 4th centile and OFC 1st centile. She had narrow palpebral fissures, retrognathia, hypoplastic toenails, marked joint hypermobility, especially distally, and a hirsute back.

Cranial MRI demonstrated reduced white matter bulk with a small pons.

Patient 11

This 15 year old child is the son of non-consanguineous parents. Neither the parents nor siblings exhibit a phenotype similar to the patient's.

He was born at 40 weeks' gestation following an uncomplicated pregnancy. There were no neonatal complications, and no feeding difficulties.

Developmental milestones have been delayed, especially speech. Independent walking was achieved at 13 months, and his first words were at 2.5 years.

At the age of 15 years, he had moderate ID and attended a special school. He had autistic spectrum disorder and attention deficit hyperactivity disorder, and he displayed some aggressive behaviours. He was prescribed risperidone and methylphenidate.

He had epilepsy with generalized and absence seizures as a child, although this has resolved and he no longer requires anti-epileptic medication. He also had discoloured teeth with poor enamel formation, and required four extractions for dental caries. He also had bilateral strabismus.

Physical exam at age 15 years demonstrated height 164.3cm, weight 74.8kg and OFC 58cm. Facial dysmorphism included prominent eyebrows, deep-set eyes, hypoplastic alae nasi and wide mouth. He also had pes planus, achilles tendon contracture and hypoplastic nails.

Previous testing including FMR1 repeat length and sequencing of the *RAI1* gene was normal.

Patient 12

This 17 year old is the son of non-consanguineous parents. His mother and two sisters were thought to have a similar phenotype. He was born at 42 weeks' gestation after an uncomplicated pregnancy. Birth weight was 3.2kg. He required five days in Special Care.

Developmental milestones have been delayed, with the patient walking independently at 2.5 years age. His first words were between 4-5 years. He had ID, with special educational needs. He was in simple employment following school. He had attentional difficulties, and displayed some aggressive/angry behaviours.

Physical exam at age 17 years demonstrated OFC 53.8cm. He was not thought to be facially dysmorphic.

Patient 13

This 22 year old female is the sister of patient 7. She was born at 34 weeks' gestation after an uncomplicated pregnancy. Birth weight was 3.28kg. There were no neonatal complications.

Developmental milestones have been delayed, with the patient walking independently at 18 months and developing first words at approximately four years of age. She had ID with special educational needs and was

attending college after school. She had attentional difficulties and displayed some aggressive/angry behaviours.

Physical exam at age 22 years demonstrated height 167cm, weight 65kg and OFC 54.6cm. She had joint hypermobility.

Patient 14

This 20 year old female is the sister of patients 7 & 8. She was born at term after an uncomplicated pregnancy. Birth weight was 3.03kg. There were no neonatal complications.

Developmental milestones have been delayed, with the patient walking independently at four years of age. Her first words were at approximately four years of age. She had ID with special educational needs. She was working in retail after school. She had some aggressive/angry behaviours.

Physical exam at age 20 years demonstrated height 165cm, weight 60kg and OFC 54cm. She had joint hypermobility.

Patient 15

This 47 year old female is the mother of patients 7-9. She had special educational needs and had epilepsy as a child. Physical exam at the age of 47 years demonstrated height 164cm, weight 60kg and OFC 54cm.

Patient 16

This 2-year-old male is the child of non-consanguineous parents. Neither the parents nor siblings exhibit a phenotype similar to the patient's.

He was born at 39+2 weeks' gestation following an uncomplicated pregnancy. Prenatal scans demonstrated accelerated growth, but no other abnormalities were detected. Birth weight was 4735 g. He had feeding difficulties early in life, with bottle feeding taking > 1 hour.

His development has been delayed, with social smile developing at 8 weeks, independent sitting at 16 months, and walking not yet achieved by age 29 months. Speech is also absent at 29 months. Evaluation by Bayley-III-NL showed a developmental age of 11 months at calendar age of 26 months. Behaviorally, he exhibits automatisms, mouthing, body rocking, drooling, breath holding spells, and outbursts of screaming. He does not make eye contact.

This patient also has significant neurological abnormalities, including benign external hydrocephalus. Initially, he exhibited hypertonia and hyperreflexia of the extremities and later developed mild axial hypotonia. He has dystonic movements of the hands and choreatic movements of the arms. Cardiac evaluation showed no structural abnormalities.

Physical exam at age 2 years and 5 months showed a height of 95 cm, weight of 15 kg, and OFC of 52 cm. Dysmorphic facial features include plagiocephaly, broad forehead, small eyes, downturned corners of mouth, uplifted right earlobe, and chin dimple. Physical exam also demonstrates strabismus convergens, rotary nystagmus, a sacral dimple, and pedes planovalgus.

Previous investigations included Sanger sequencing of *L1CAM*, *FMR1* repeat length analysis, and chromosomal microarray, none of which revealed any abnormalities.